North Sea seaweeds: DIP and DIN uptake kinetics and management strategies

Lubsch, Alexander

Chapter 7

'Manual for nutrient uptake kinetics in seaweed cultivation'

The studies in this thesis were conducted with clear fundamental and applied objectives to aim for a better understanding of the ecophysiology of the 4 seaweeds and at the same time contribute to applications and implications in the development of a new bio-based economy emerging in the Western hemisphere, and in particular around the North Sea area. In the following chapter specific examples on the implementation of our results into seaweed operations, such as offshore cultivation, tank cultivation, IMTA applications and bio-filtration activities are given. Generally, our data on uptake kinetics and nutrient management (Chapter 2, 4 & 6) can perfectly well be integrated into dynamical models, such as ERSEM (European Regional Seas Ecosystem Model) to project the effects of environmental variables on growth and composition in seaweed species. Dynamical models allow not only an estimation on growth of seaweed and its composition, for example, the seasonal growth and composition of *S. latissima* (Broch & Slagstad 2012) and its potential production in seaweed farms (for the North Sea and UK coastal waters: van der Molen et al. 2018), but also support to project the economic feasibility of sustainable seaweed production (for the North Sea area: van den Burg et al. 2016).

7.1 Offshore cultivation

Offshore cultivation of seaweed opens opportunities for large scale operations to produce food, feed and chemical compounds for further utilization (McHugh 2003, Bartsch et al. 2008, Holdt & Kraan 2011, Fernand et al. 2016). Although offshore cultivation leaves relatively little control to environmental variables, some factors can be manipulated. Some structural systems for seaweed cultivation are designed to be moved vertically, thus allowing to submerge the system to depth where light conditions are favourable for growth or higher nutrient concentrations are present. In addition, a submergible system can be moved to reduce hydrodynamic impact on the seaweed and the structure itself during severe oceanic weather conditions (Buck & Buchholz 2004). During periods of low nutrient concentration in the surrounding ocean water, fertilizer can be added, for example by deploying porous containers with slow-release fertilizer (Neushul et al. 1992). Information on nutrient ecophysiology, as studied for *U. lactuca* (Chapter 2 & 3), *S. latissima*, *L. digitata* (Chapter 4), and *P. palmata* (Chapter 6) in this thesis, offer good opportunities to locate potential cultivation sites in relation to nutrient availability and manipulate nutrient additions appropriately on site, as well as adjust the frequency of additions and ratios.

The following solely serves as a hypothetical example on nutrient uptake management in *S.latissima*, as observed in our studies (Chapter 4) and demonstrates how the fundamental data on nutrient uptake kinetics and management can be applied: a suitable location, based on nutrient availabilities, to culture *S. latissima* should offer (more or less constant) nutrient concentrations in the seawater of approximately 19.5 $\mu mol \cdot L^{-1}$ DIN and 1.5 $\mu mol \cdot L^{-1}$ DIP for optimal growth at any time (see V_M in relation to nominal offered concentration; Table 8-1). A seaweed farm with a total line length of 2 km (whether long line arrangement or ring system) and monocultures of young *S. latissima*, have, for example, an averaged SA of 0.04 m^2 (400 cm^2) per meter of line. This amounts to a total SA of 800.000 cm^2 for 2 km of line. Initial daily requirement of DIN and DIP would account for 43.7 g N and 7.4 g P, according to our data on V_M in *S. latissima* (Table 8-1; Chapter 4). A daily growth of 4 %, as observed for *S. latissima* in our study and by others (Nielsen et al. 2014, Boderskov et al. 2015), increases the total SA to its tenfold in 59 days, calculated as follows:

$1.04^t = 10 \rightarrow t = \ln(10) \times \ln(1.04)^{-1}$,

with t as time (days) needed to 10-fold the SA at a growth rate of 4%. After 118 days, the initial SA has increased by the factor 100 to 4m^2 per meter of line and the total daily DIN and DIP requirements in this scenario have expanded likewise, and account for 4.4 kg N and 0.74 kg P. Fertilizers (N and P in the appropriate concentration and ratio) can be added in periods of nutrient limitations. Also, by tracking seasonal changes of DIN and DIP availability in the seawater, an estimate on the eco-physiological performance of the seaweed can be given based on the information on the ISC, and thus can reduce the necessity for tissue content analysis of N and P. The high ISC and an uncoupled nutrient uptake in *S. latissima* allows for up to 7 weeks of DIN and DIP depletion or limitations without major forfeit in growth. For example, when nutrient concentrations in the seawater show a DIN concentration of approximately 10 $\mu mol \cdot L^{-1}$ over 4 weeks, the rate of assimilation (V_M) in *S. latissima* is only half-saturated. This external limitation can be compensated by utilization of internal DIN pools. Approximately one third of the ISC for DIN in *S. latissima* would compensate for the limitation, accounting to 54.6 $\mu mol \cdot cm^2$, calculated as follows:

$$\Delta ISC = d_M \times (V_M - T_c),$$

with d_M as days of nutrient limitation, V_M as metabolic uptake rate, and T_c as limiting nutrient concentration. This estimate allows to adjust appropriate nutrient additions and duration: V_s for DIN is approximately 3 (2.9) times more efficient than V_M in *S. latissima* and a nominal DIN concentrations in the seawater of approximately 19.5 $\mu mol \cdot L^{-1}$ optimally saturates V_M. Therefore the addition of DIN to create a seawater concentrations of approximately 60 $\mu mol \cdot L^{-1}$ (56.5 $\mu mol \cdot L^{-1}$) over the next 8 (7.4) days is advisable to allow *S. latissima* to refill internal N pools in this scenario, calculated as follows:

$$d_{ISC} = \Delta ISC \times (V_S - V_M)^{-1},$$

with d_{ISC} as days needed to refill ISC, ΔISC as available capacity, V_S as the surge uptake rate, and V_M as the metabolic uptake rate. The addition of costly nutrients to establish higher concentrations is superfluous, as V_S is limiting, and hence excessive nutrients are 'washed' away in an open system and are lost for the operation.

Naturally, this over-simplified example is not able to adequately represent the complex and combined mechanisms of many environmental factors impacting physiology, growth and composition of a seaweed. However, suitable nutrient uptake and nutrient management data for the 4 species used during this thesis is very scarce, and our data can be integrated into dynamic ecological models, such as ERSEM for a more detailed projection and can support economic (feasibility) studies on seaweed farming and can be used for studies of resulting impacts on marine ecosystem services. An approach in seaweed farming is a multi-layered poly-culture of seaweed (Reith et al. 2005), that is, different groups of seaweed have differing light requirements, so that green, brown, and red seaweeds can be grown at various depths, allowing for integration of crops through a layered growth of different seaweed groups. The nutrient demand of poly-cultures, for example *S. latissima* integrated with *P. palmata*, can be evaluated accordingly using the data presented in this thesis. Poly-cultivation is also a promising approach for IMTA activities, also related to bioremediation purposes and/or ecosystem services. In ecosystem services, coastal seaweed farms can act as the ultimate barrier to (re-)capture/recycle dissolved phosphate, before it is diluted to the deep sea. Phosphorus is often a limiting nutrient in (terrestrial) agriculture, due to its low availability and mobility in soils. Soil fertility has been identified as one of the most significant challenges in achieving food security in the developing world (Elser 2012).

7.2 Integrated multi-trophic aquaculture (IMTA)

In most cases the stimulus for the integration of seaweeds in a multi-trophic aquaculture is based on cleaning the seawater from increased concentrations of dissolved nutrients, originated from fish farm activities, and by this prevent eutrophe conditions (Troell et al. 1997). Most fish farms are located close to the coastline, typically in sheltered bays and estuaries, which are vulnerable ecosystems with regard to eutrophication. Several IMTA systems, including multi-layered cultures of different seaweed species, have been proposed to combine seaweed cultivation and fish farms in order to mitigate effects of eutrophication and at the same time produce valuable biomass for food, feed and fertilizer (Reith et al. 2005). It is important to note that fish (by definition) produce organic waste, and seaweeds (by definition) prefer the inorganic form of nutrients. Organic nutrient sources can be differentiated from inorganic nutrients in that they must be decomposed/re-mineralised before they are available to the seaweed. This is done by microorganisms such as bacteria. Moreover, large fractions of the waste produced by fish are in particulate material. This is a mismatch from the beginning. Filter-feeders may be much more efficient in removal of waste products in the immediate vicinity of fish farms (e.g. Soto & Mena 1999, Mazzola & Sarà 2001). The extreme enrichment of N and P in the water column deriving from fish farms (Aure & Stigebrandt 1990, Gowen 1994), paired with their continuous operation throughout the year, and often surrounding (natural) restrictions of available space, require an efficient and yearlong functioning approach.

In our studies, *S. latissima* and *P. palmata* showed the highest uptake rates for both, DIP and DIN with a ratio of 1:13, respectively 1:10, and growth rates of 3 % d^{-1}, respectively 1 % d^{-1} (Table 8-1), which makes them promising specimen for multi-layered poly-cultures in close vicinity to fish aquaculture. The constant nutrient uptake by *S. latissima* (Chapter 4) and the oscillating nutrient uptake, as well as the strong dependency on available DIP for an increased DIN uptake in *P. palmata* (Chapter 6) complement each other in DIP and DIN removal. In terms of bioremediation, this poly-culture could represent the 'main body' of the seaweed cultures, with supplementary configuration on size and integration of other species in correspondence to efficiency, nutrient removal ratios of DIN and DIP, as well as season. Downstream the IMTA operation, nutrient concentrations (and ratios) should be on the natural level, to minimize effects on the ecosystem. A DIN limitation in the seawater can create a downstream gradient with a high DIP to DIN ratio. According to our results, the integration of *L. digitata* with a DIP:DIN-uptake ratio of 1:8 under V_M (Table 8-1; Chapter 4) could help to diminish such a gradient. A DIP limitation, creating a high DIN concentration in the downstream could be mitigated by the integration of *U. lactuca*, which showed the lowest DIP uptake rates and considerably high DIN uptake rates (Table 8-1). Another possibility would be the harvest of *P. palmata* and replacement with *S. latissima*. As our study showed, *P. palmata* strongly depends on the availability of DIP to increase DIN uptake, in order to increase the total dissolvable protein concentration, hence the nutritional value (Chapter 6). Its replacement by *S. latissima* with a DIP to DIN uptake ratio of 1:13 under V_M (Table 8-1; Chapter 4) could scale down the forming of a high DIN concentration under DIP limitation. During the summer months, *S. latissima* and *P. palmata* can/will reduce their growth due to an increase in water temperature (discontinued growth in *S. latissima*: >20 °C (Pedersen 2015); in *P. palmata*: >18 °C (Morgan & Simpson 1981)), and an integration of *U. lactuca* (>25 °C Fortes & Lüning 1980) could complement the poly-culture on the surface layer to maintain an efficient bioremediation, produce biomass, as well as shade and protect *S. latissima* and *P. palmata* in deeper layers from harmful light intensities. It is also conceivable that an integration of *U. lactuca* could divert certain (meso-) grazers to minimize feeding impact on *P. palmata* and *S. latissima* from spring to autumn. Generally it is assumed that fast growing seaweeds with filamentous texture can reduce the impacts on herbivory by escaping consumers in space, time or high growth rates (Lubchenco & Gaines 1981). However, seaweed-herbivore interactions and feeding preferences on different seaweed species represent a largely unexplored facet of seaweed ecology (e.g. Paul et al. 2006, Toth & Pavia 2007, Molis et al. 2015).

In spite of the many opportunities as described above, major fundamental challenges remain for IMTA: in addition to a possible mismatch of organic versus inorganic nutrients and the large fraction of particulates in waste from fish, the balance of waste stream and size of seaweed site, as well as position, is difficult. Large seaweed populations are required to absorb the dissolved waste of large quantities of fish, and the other way around. In practice this will be difficult to match.

7.3 Tank cultivation

In tank cultivation of seaweeds, it is possible to control (almost) all environmental factors and thus knowledge of the ecophysiology is very important to maximise growth rates, yields, and/or acquire desired products. Information on the optimum nutrient requirements in order to calculate the nutrient supply rate to a tank are essential. This includes not only knowledge on V_S and V_M for DIN and DIP of the applied species, but also information on the optimum nutrient uptake ratio to determine the most economical additions of for example macro- or micro-nutrients (Harrison & Hurd 2001). In addition, information on the ISC can help to mitigate and control the potential entry of epiphytes and/or microalgae. By starving the seaweed for a suitable time period, before the ISC for DIN and DIP have decreased to critical levels, the growth of epiphytes could be hampered, respectively controlled in cultivation practices (Pickering et al. 1993). The longer the period of time before the ISC of the cultivated species is depleted, the better the control of contamination by epiphyte and/or microalgae. Typically, V_S of nutrient starved seaweed is multiple times greater than V_M (Conway et al. 1976) and hence the seaweed is able to quickly overcome its nutrient deficiency, after the addition of saturating nutrient concentration. For example, a tank monoculture of *P. palmata* with a total SA of 10.0 m^2 (100,000 cm^2) incubated in a 4000 L aerated cultivation tank, constant water temperature of 12 °C and a 16/8 h light-dark rhythm with a light supply of 70 µmol photons $m^{-2} \cdot s^{-1}$ would require a daily addition of 7.8 g N and 1.8 g P, calculated as follows:

$n = m/M \rightarrow m = n \times M$,

with n as the daily amount of substance (or chemical amount, or daily requirements by the seaweed), M as the molar mass (N=14 $g \cdot mol^{-1}$; P=31 $g \cdot mol^{-1}$), and m as the required mass of N, respectively P.

$m_N = 0.56 \text{ mol} \times 14 \text{ g} \cdot \text{mol}^{-1} = 7.84 \text{ g}$

$m_P = 0.057 \text{ mol} \times 31 \text{ g·mol}^{-1} = 1.76 \text{ g}$

Naturally, N and P are only available as chemical compound and the required concentrations of N and P can be added, for example, in the forms of potassium nitrate (KNO_3; 101.1 $g·mol^{-1}$) and potassium-dihydrogen-phosphate (KH_2PO_4; 136.1 $g·mol^{-1}$). In this case, the daily additions would amount to 56.6 g KNO_3 and 7.8 g KH_2PO_4. After addition, the initial DIN and DIP concentration in the seawater inside a 4000 L tank, should have a DIN concentration of 140 $\mu mol·L^{-1}$, respectively 14.25 $\mu mol·L^{-1}$ (N:P-ratio of 1:10, neglecting potential background concentrations already present in the seawater), calculated as follows:

$c_x = n_x \times V^{-1}$,

with c_x as the concentration, n_x as the total daily molarity required, and V as the volume of the tank filled with seawater. Due to an oscillating nutrient uptake by *P. palmata* in a weekly rhythm (Chapter 6), a weekly pulse of approximately 400 g KNO_3 and 100 g KH_2PO_4, rather than a constant daily supply, would be ideal in this scenario. Simultaneously, the weekly nutrient pulses will result in starvation of epiphytes- and/or microalgae between the nutrient additions. A weekly addition of 400 g KNO_3 and 100 g KH_2PO_4 would create an initially very high DIN and DIP concentration of 980 $\mu mol·L^{-1}$, respectively 100 $\mu mol·L^{-1}$ in the cultivation tank. The high uptake rates of *P. palmata* under V_S and V_M conditions (Table 8-1) are capable of refilling the ISC in a short time under these saturating nutrient conditions. The weekly dosage can be adjusted to growth rates. An averaged growth rate of 1 % d^{-1} has often been reported for *P. palmata* (Sanderson et al. 2012, Corey et al. 2014), similar numbers are reported in this thesis (Chapter 6). A growth rate of 1 % d^{-1} is calculated as follows:

$f(t) = a \times (1 + p·100^{-1})^t$,

with *a* as the total initial SA, p as growth rate, and t as the number of cultivation days. Accordingly, the total SA of the *P. palmata* culture will increase from 10 m^2 to 11.5 m^2, after 2 weeks (f(t) =

$100{,}000\ cm^2 \times (1 + 1/100)^{14} = 100{,}000\ cm^2 \times 1.01^{14} = 115{,}000\ cm^2$). In tank cultivation of *P. palmata*, it is advisable to regularly control DIP concentration in the water and ensure DIP availability, due to the strong necessity for elevated DIN uptake and thus growth (Chapter 6). When young sporophytes of *S. latissima* are cultivated in tanks, it is not advisable to pulse extremely high nutrient concentrations, as shown in the study in Chapter 4: all sporophytes (n=7) exposed to daily high DIP and DIN pulses perished within 3 weeks.

7.4 Biofiltration

In order to predict the efficiency of a particular seaweed in water treatment facilities (biofilters), for example in land-based tank systems or in *situ* applied biofilters at inlets of cooling water for power plants, information about uptake kinetics are indispensable and can help to control effluent and productivity in environmentally responsible practices (e.g. Robertson-Andersson et al. 2008, Copertino et al. 2009). The opportunistic seaweed *U. lactuca* has been identified as a promising species in biofilter systems and in IMTA systems (e.g. Cohen and Neori 1991, Neori et al. 2003). The majority of studies related to the efficiency of N and P removal from seawater have been conducted under field conditions (Cohen & Neori 1991, Naldi & Viaroli 2002, Neori et al. 2003) and verify the feasibility of *U. lactuca* for biofiltration, but a quantification on total filtration (or nutrient assimilation) capacity was unknown. Our results on the uptake kinetics support that *U. lactuca* can efficiently be applied in biofiltration systems for an excess nutrient uptake, leading to less eutrophic waters (Chapter 2). Despite the quickly filled ISC and the corresponding declines in nutrient uptake rates of approximately 90 % for DIP and 80 % for DIN in saturating concentrations, V_M in *U. lactuca* can still significantly contribute to the reduction of nutrient loads. Although our results on nutrient uptake kinetics showed higher uptake rates in the perennials *S. latissima* and *P. palmata,* higher growth rates (Table 8-1), as well as a greater SA (to take up nutrients) in relation to biomass favor the opportunistic *U. lactuca* to be employed in filtration systems. The correlation factors presented in this thesis for SA with FW and DW in *U. lactuca* (Chapter 2) enables conversions between these standardization units. This allows for an accurate estimation of the efficiency and sustainability of a (large scale) bio-filtration system, as industrial enterprises typically determine the FW rather than SA for practical reasons. Moreover, efficiency and sustainability of these biofilter systems can be maintained by controlling the effluent and/or adapting the biomass, also in accordance to growth rates. In the case of *U. lactuca*, for example, a bio-filtration system containing a biomass of 100 kg FW would be an equivalent SA of approximately 7.7 million cm^2, given our conversion factor for FW (y=0.013x). This SA could take up a total of around 538,000 μMol DIP and 17.7 million μMol DIN in a day, according to the uptake rates under V_M for DIP and DIN in *U. lactuca* (Table 8-1). The daily reduction of DIP and DIN from the seawater by *U. lactuca* corresponds to 16.7 g of P and 250 g of N, calculated as follows,

$n = m/M \rightarrow m = n \times M$,

with n as the amount of substance, *M* as the molar mass (N= 14 $g \cdot mol^{-1}$; P= 31 $g \cdot mol^{-1}$) and m as the mass. A moderate growth rate for *U. lactuca* of averaged 4 % d^{-1}, as observed in this thesis (Chapter 2), would double the initial biomass of 100 kg FW in approximately 18 days, according to an exponential increase, determined by the following function:

$t_2 = \ln(2) \times \ln(1.04)$,

with t_2 as doubling time, ln(2) as the logarithm for doubling the biomass and ln(1.04), resembling the daily growth rate of 4 %. However, this example on the quantification of the bio-filtration capacity by *U. lactuca* requires optimal mixing of the water column for nutrient distribution and constant biomass circulation to avoid self-shading and provision of light for each individual. Related to growth rates, the filtration tanks should offer sufficient room for the increase in biomass (Figure 7-1).

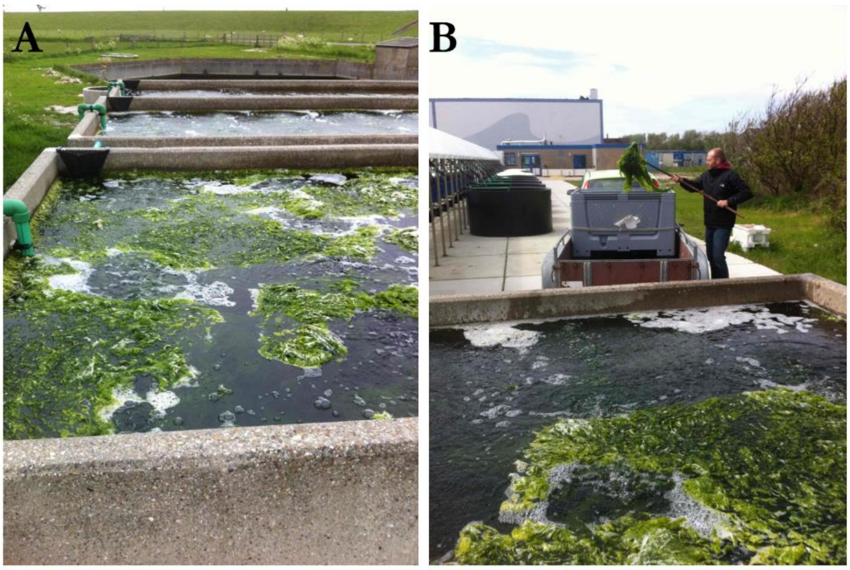

Figure 7-1. Carrying capacity. (A) *Ulva lactuca* piling up to '*Ulva*-bergs' in a bio-filtration tank and (B) harvest of piled up biomass with a pitchfork at the NIOZ Seaweed Research Centre on Texel in summer 2015.